

# Precision of age estimation in red snapper (*Lutjanus campechanus*)

Robert J. Allman\*, Gary R. Fitzhugh, Karl J. Starzinger, Robert A. Farsky

National Marine Fisheries Service, Southeast Fisheries Science Center, 3500 Delwood Beach Road, Panama City, FL 32408-7403, USA

Received 7 July 2004; received in revised form 17 December 2004; accepted 21 December 2004

## Abstract

The validation of aging methods is an important step in estimating growth and longevity and has been accomplished for red snapper. However, routine age interpretation remains largely subjective. A reference collection of 300 red snapper otoliths was circulated among seven external laboratories in the Gulf of Mexico region to compare ages against our internal laboratory determinations. A precision benchmark of average percent error (APE; estimate of precision)  $\leq 5\%$  has been suggested for moderately long-lived species such as red snapper. APE ranged from 2.5 to 6.0% for six facilities with no apparent bias in estimates as age increased. One initial estimate was notably higher with an APE of 11.6% and bias was evident. Beyond the need for initial training to recognize annulus patterns in decades-old fish, common differences between readers were related to interpretation of the otolith edge type and to interpretation of the first annulus. Precise measurement of annulus distances and identification of otolith edge patterns aided by light reflectivity measurements indicated that annual rates of transition from translucence to opacity were consistent. However, annual differences in the seasonal timing of otolith zone transition occurred on the order of a few months (this study and others). The degree of opacity varied in the first annulus, but the distance from the core to the distal edge of the first annulus was consistently around 1 mm (mean = 1.05 mm; S.D. = 0.11). By recognizing possible variations in these patterns and with use of periodic reader training and a reference collection, our expectation is that a 5% APE precision target can be readily achieved and improved upon.

Published by Elsevier B.V.

**Keywords:** Red snapper; Otolith; Average percent error; Annulus; Marginal increment analysis

## 1. Introduction

Red snapper, *Lutjanus campechanus* (Poey, 1860) is one of the most economically important fish species in the southeastern U.S. and has been the subject of several age and growth studies (Futch and Bruger,

1976; Bortone and Hollingsworth, 1980; Nelson and Manooch, 1982; Manooch and Potts, 1997; Patterson et al., 2001; Wilson and Nieland, 2001). While validation of aging methods is an important criterion in estimating growth and longevity, and has been accomplished for red snapper (Baker and Wilson, 2001), age interpretation remains largely subjective. The repeatability of age estimates (i.e., precision) is an important measure to compare the proficiency of different otolith readers and to measure individual drift over time (Campana,

\* Corresponding author. Tel.: +1 850 234 6541x206; fax: +1 850 235 3559.

E-mail address: [bob.allman@noaa.gov](mailto:bob.allman@noaa.gov) (R.J. Allman).

2001). Routine annual aging is increasingly performed to track recruitment and age structure trends over time for stock assessment purposes (Allman et al., 2002). Precision of routine age determinations (e.g., from reference samples aged by different readers) ultimately reflects an ability to distinguish strong from weak year classes and is therefore an important concern for assessing stock condition (Beamish and McFarlane, 1995; Crone and Sampson, 1998; Campana, 2001).

Evidence from aging workshops and initial exchanges of red snapper otoliths between laboratories indicate that differences between readers affecting estimates of precision are most often related to interpretation of the otolith edge type and to interpretation of the first annulus (Allman et al., 2002). These two factors have also been noted for other species and are often problematic as standardized age-structure interpretations become more important for production aging (Francis et al., 1992; Fowler, 1995; Campana, 2001). These problems are most acute when errors of one or two years in age-class assignment occur in the most common age classes (i.e., 2–5 years). These errors can mask clear determination of year-class strength—the “smearing effect” (Beamish and McFarlane, 1995).

Our first objective was to compare red snapper ages from readers at our facility to seven other Gulf of Mexico laboratories using a reference collection. Secondly, we attempted to resolve the edge and first annulus interpretation problems and to reconcile potential differences among studies by: (1) determining the timing of opaque zone formation using the method of marginal increment analysis (MIA) based on carefully selected, sectioned and measured sagittal otolith samples with broad geographic and seasonal representation. We then compare the results from the selected otoliths with those of other red snapper age studies; and (2) by measuring the otolith core-to-edge distances from seasonally-collected juveniles through the period of their first annulus formation in order to characterize the shape, appearance and location of the year-one annulus.

## 2. Methods

Expanded sampling of red snapper otoliths from the directed fishery during 1998–2000 in the U.S. Gulf of Mexico allowed us to draw a large number of otoliths

from all the gulf states and from many different age classes. Otoliths were sectioned and mounted for aging following the methods of Cowan et al. (1995). We designate these as adults even though we later show these otoliths often represent relatively young fish (>1 year in age). We also obtained otoliths from fish, which we designate as juveniles (age  $\leq 1$  year) captured during fishery independent surveys from February to November 2002. Otoliths were extracted from the juveniles and prepared in a manner similar to the adult specimens but using a low-speed (90 rpm) sectioning saw instead of the typical high-speed (1800 rpm) saw used for adult otoliths.

Sectioned otoliths were assigned an age based on the count of annuli (opaque zones observed with reflected light) along the dorsal edge of the sulcus acousticus and on the degree of marginal edge completion. For example, otoliths were advanced 1 year in age after 1 January if their edge-type was a nearly complete translucent zone. Typically, marine fish off the southeastern United States complete annulus formation (opaque zone) by late spring to early summer (Beckman and Wilson, 1995; Patterson et al., 2001; Wilson and Nieland, 2001). Therefore, an otolith with two completed annuli and a large translucent zone would be classified as age 3 if the fish was caught during spring in expectation that a 3rd (opaque) annulus would have soon formed. After 30 June when opaque zone formation is typically complete, all fish were assigned an age equal to the annulus count by convention. Thus, an annual age cohort was based on a calendar year rather than time since spawning (Jearld, 1983; Vanderkooy and Guindon-Tisdell, 2003).

### 2.1. Reference collection

A reference collection of 300 adult red snapper otolith sections was selected to represent most age classes, all seasons, both sexes, different collection years, good to poorly prepared otolith sections and the entire geographic range sampled (Campana, 2001). Prior to exchanging and aging the reference collection, a training image set was created for distribution via compact disk to all gulf laboratories by digitally photographing 100 otolith sections. Otolith sections for the training image set were selected using the same criteria as for the reference collection. In order to establish the location of opaque zones for the training

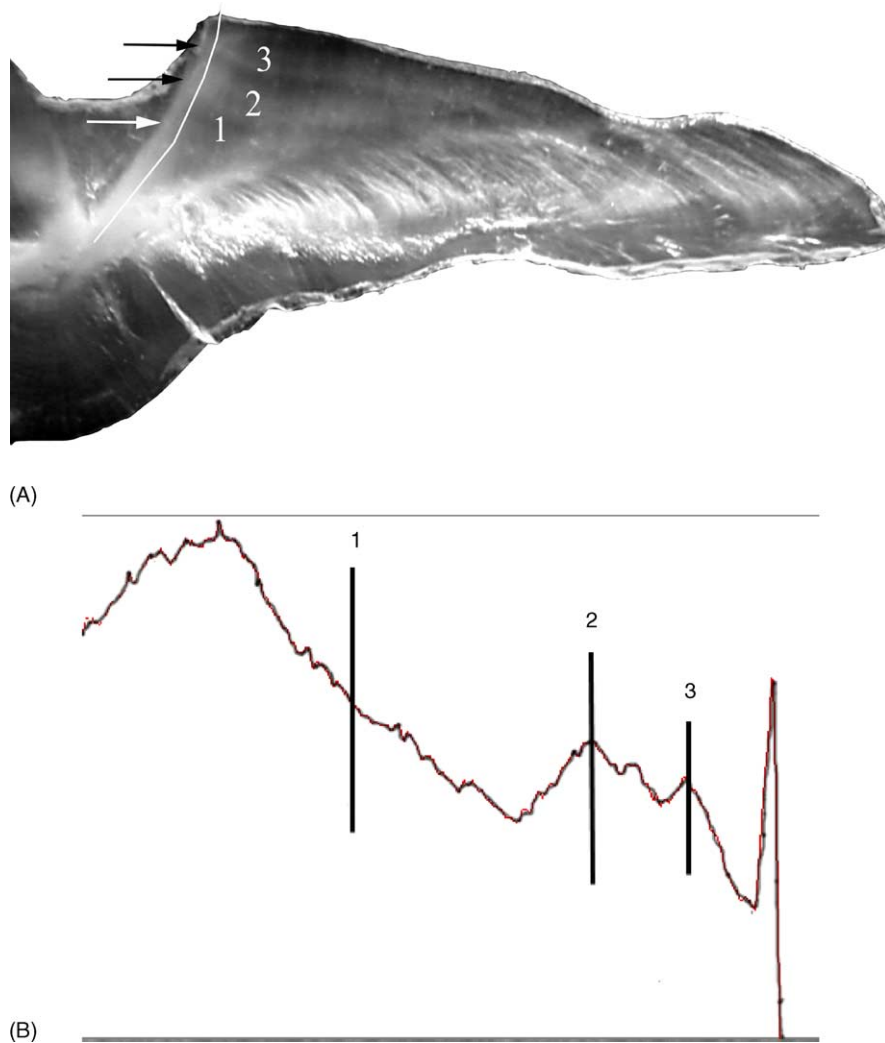


Fig. 1. (A) Measurement path for selected otolith sections and (B) reflected red spectrum light intensity curve for the measurement path. Number 1 = core to distal edge (as measured by the midpoint of the declining slope of the first annulus), numbers 2 and 3 indicate peak opacity for the second and third annuli, respectively.

manual images, our laboratory and another laboratory (laboratory 2), both of which had previous experience aging red snapper, developed a consensus on annuli location. Other participating laboratories had little or no experience aging red snapper. Each otolith section was digitally photographed twice, once without any annotation and once with assumed annuli marked. The otolith training image set was distributed to all laboratories and afterward an otolith training workshop was held to address any questions.

Once a general consensus on age assignment was achieved by all laboratories on the training image set, the reference collection was exchanged. The reference collection was first used to address within-laboratory reader variation and to establish a consensus age set for each laboratory. These consensus age sets were then compared between laboratories. Reference collection ages from seven external laboratories were compared to those generated by our laboratory since our laboratory had had notable experience and was initiating

the reference collection. Average percent error (APE; Beamish and Fournier, 1981) was used to compare our age determinations to those of other laboratories. We considered an APE of 5% as a reference point for moderately long-lived species with relatively difficult to read otoliths (Morison et al., 1998; Campana, 2001). Age bias plots were used to look for systematic differences between laboratories for reference collection ages (Campana et al., 1995).

## 2.2. Optical analysis

We selected over 1000 adult red snapper otoliths from 1998 to 2000 covering all months and from all five gulf states. A second selection of 259 from this collection was made to retain those sections which were precisely cut through the core at an angle perpendicular to the anterior/posterior axis to locate a consistent point of origin. We took care to select only those sections that did not show edge damage, preparation flaws, or other defects typical of routine age preparations.

Each otolith section was examined at  $25\times$  magnification and measured using an image analysis system (PhotoShop 6.0<sup>®</sup> equipped with the Andromeda Measurement Filter, used for calibration). Measurements to the nearest 0.01 mm were made for each of the following characteristics: core to the distal edge of the first opaque zone, core to the center of the second opaque zone, core to center of third zone and any subsequent opaque zone, and core to edge. The measurement path extended from the core to the edge along the dorsal side of the sulcus acusticus (Fig. 1A). Opaque zones and edge structure were selected by eye and verified using light intensity curves. The associated light intensity curve represented the red spectrum along that path. The red spectrum was used for the wavelength profiles because it served to increase resolution and helped to reduce subjectivity by using light patterns to aid in measurements. Since the first opaque zone is usually broad and diffuse, we chose to measure to the distal edge of the first zone instead of the center of the zone as we did for the other opaque zones. The distal edge is seldom a discrete point but a gradual transition to translucent, so we used the midpoint of the declining slope of the light intensity curve to aid in locating the edge and to reduce subjectivity (Fig. 1B). Measurements were made without prior knowledge of collection date.

Since we count opaque zones as annuli, a full annual cycle should include two successive points of maximum opacity. However, the marginal increment is defined as the distance across the translucent zone measured from the last opaque zone (maximum reflected light) to the otolith edge. Our preliminary observations revealed that as the translucent marginal increment increased approaching the next season's opaque zone, the reflected light also increased. Often some opacity was apparent to the eye before the annual cycle was complete on the margin. Since the increase in opacity was gradual, it was extremely difficult to judge the maximum value until the edge was sufficiently distinct (i.e., opaque) to evidence a decline in reflected light. Accordingly, the edge values were seldom equal to zero.

While marginal increment analysis (MIA) may not be the best method for validation, it does allow insight into timing of otolith zone formation (Campana, 2001). MIA can be divided into edge analysis (frequency of edge type) or an increment measurement approach, the latter being the more common method (Campana, 2001). We will refer to these MIA approaches as edge-frequency and edge-measurement analyses, respectively. We employed both edge-measurement and edge-frequency analysis, but compare our findings with edge-frequency results of others. Least squares linear regression was used to examine the relationship between juvenile total length (TL mm) and core to edge otolith distance (mm). Data were natural log transformed to meet the assumptions of homogeneity of variance and normality.

## 3. Results

### 3.1. Reference collection

The APE for the three otolith readers from our laboratory was 2.5% for the reference collection. Outside laboratories reported from one to four otolith readers per laboratory. Those laboratories which had more than one reader and reported an internal APE, had APE values of 3.89% (four readers), 5.02% (three readers) and 11.11% (two readers). Initial comparisons of our ages to external laboratory ages indicated that laboratories 1–4 had an APE below the 5% target (2.8, 3.5, 3.7 and 4.5%, respectively) and laboratories 5 and 6 had an APE slightly above (5.9 and 6.0%, respectively)

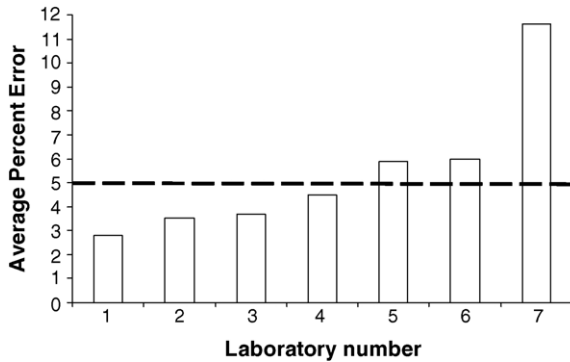


Fig. 2. Average percent reader error (APE) for seven external laboratories compared to internal laboratory ages from the 300 otolith reference collection. Dashed line indicates the 5% APE reference point.

(Fig. 2). However, major differences were noted between the internal readers and laboratory 7 readers (APE of 11.6%). Laboratory 7 also recorded the highest APE for internal readings (11.11%). To determine if age differences between internal and laboratory 7 readers were systematic, an age bias plot was used to compare the mean laboratory 7 age to each of the age categories for internal reader ages. We noted that laboratory 7 tended to overage younger fish (ages 1–5) and underage older fish (>age 8; Fig. 3). In contrast, an age bias plot of mean laboratory 1 age versus internal age categories indicated good agreement especially for the most common age classes (i.e., ages < 8; Fig. 4). After investigation we determined that laboratory 7 had enlisted a relatively new otolith reader. Laboratory 7 also

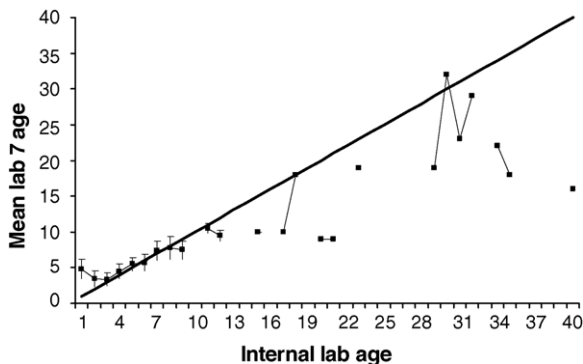


Fig. 3. Age bias plot of mean laboratory 7 age for each age category of internal laboratory age  $\pm$  one standard deviation. Solid line indicates a 1:1 equivalence.

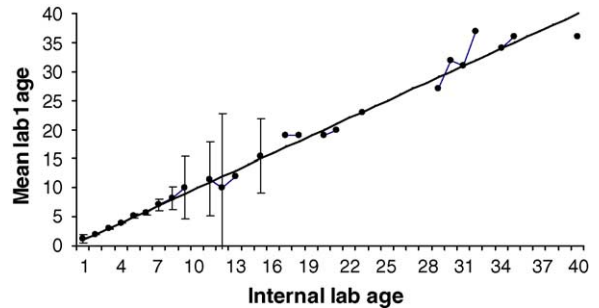


Fig. 4. Age bias plot of mean laboratory 1 age for each age category of internal laboratory age  $\pm$  one standard deviation. Solid line indicates a 1:1 equivalence.

prepared their own laboratories' otolith sections using a slightly different method compared to our method which might have led to some of the discrepancies. To help clarify discrepancies, digitized otolith images from the reference collection were exchanged between our laboratory and laboratory 7. Once the main discrepancies were resolved, a second reference set of 300 randomly selected otoliths was selected from the laboratory 7 collection and read by both laboratories. An APE of 7.6% was calculated for this second collection.

### 3.2. Optical analysis

Two hundred fifty-nine adult red snapper otoliths that had been precisely sectioned through the core were selected, aged and measured. The samples represented all gulf states from 1998 to 2000 but were obtained primarily from Florida and Louisiana during 1999 (Fig. 5A and B). Otoliths were from all months except December. Fish having three and four opaque zones dominated the selected samples, which is typical of the age-structure in commercial and recreational fisheries (Allman et al., 2002) (Fig. 5C).

Translucent zones narrow as fish age, this can lead to differences between age classes in the pattern and timing of marginal increment formation (Smith and Deguna, 2003). Even with our relatively large sample size we needed to combine fishes of different age classes to have sufficient numbers to examine the seasonal pattern of annulus formation. Therefore, we had to assume that the annual timing of the edge minima is similar for each age class in our collection. A plot of edge distance by month of the collection indicated that the minimum marginal increment occurred April

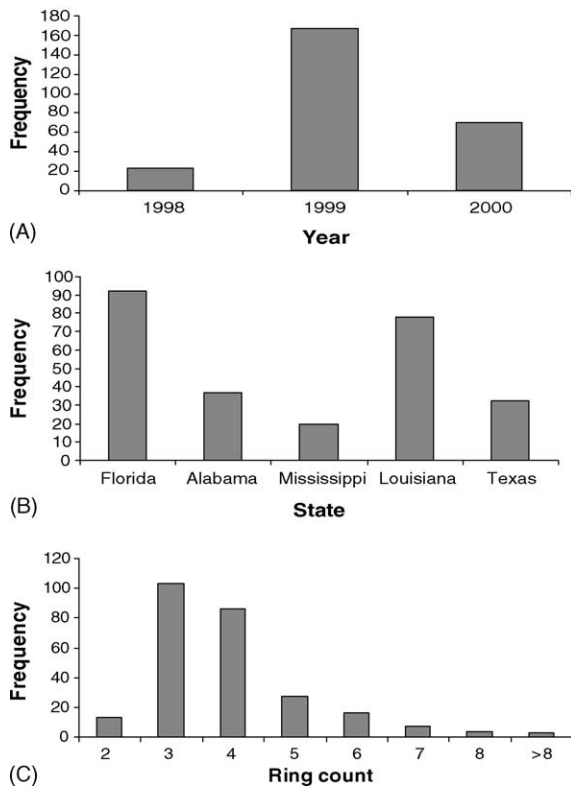


Fig. 5. Frequency of adult otolith samples selected for marginal increment analysis by (A) year, (B) state and (C) opaque ring count.

through July (Fig. 6). This same pattern was noted when we grouped age classes into two groups. We observed that the pattern appeared to be one of rapid increase in opaque zone completion (hence transition to minimal marginal increment) from March through May. Opaque margins were only observed from April to August with the highest percentage of opaque margins recorded in May. Opaque zone formation was completed relatively quickly, with frequency declining through August. Marginal increments indicated a gradual increase in the width of the translucent zones from late summer through winter (Fig. 6).

Examination of topologic features indicated that the first year's growth was visually detectable as a broadly diffuse opaque zone of similar dimension among the otoliths. This characteristic broad first opaque zone occurred on the dorsal side of the sulcus acusticus and typically had a triangular shape (Figs. 7 and 8).

To help verify our interpretation, we examined measurements obtained from 167 juvenile red snapper collected through the period of first annulus formation and ranging in total length (TL) from 33 to 241 mm (Fig. 9A). Core to edge distances (mm) were strongly related to fish total length (mm) ( $p < 0.001$ ), and the edge of the first annulus appeared at a distance of approximately one millimeter from the core (mean = 1.05 mm, S.D. = 0.11 mm). The first annulus generally formed between 110 and 170 mm TL (Fig. 9B). Otoliths from fish greater than 180 mm TL were found to have completed the broad first opaque zone. Examination of juvenile by capture date indicated that individuals collected early in the year (day 38, February) had yet to complete an annulus, while about 20% of spring caught fish (day 113–130, April–May) had completed an annulus (Fig. 9C). Most fish greater than 100 mm TL had completed annulus formation by summer (day 168–197, June–July). Summer caught individuals less than 100 mm TL were probably young-of-the-year. Fish collected in the fall (day 324, November) had all completed an annulus.

We did observe biological variation (e.g., Fig. 10) and, during initial routine processing, otoliths were not always sectioned precisely in a transverse fashion through the core. Both of these sources of variation sometimes rendered discrimination of the first annulus difficult.

## 4. Discussion

### 4.1. Reference collection

Inconsistent interpretation is a major problem for production aging laboratories. Red snapper have been relatively difficult to age based on levels of precision determined from this study and from previous studies (Allman et al., 2002). An observed trend for red snapper, commonly seen in production aging laboratories, is that within-laboratory reader estimates were more precise than between-laboratory estimates (Allman et al., 2002). This study reported precision estimates of 2.8–11.6% APE for seven external laboratories compared to internal reference collection ages, with only one laboratory greatly exceeding the benchmark 5% APE. The high value for that laboratory (APE = 11.6%) was thought to be due to a relatively inexperienced



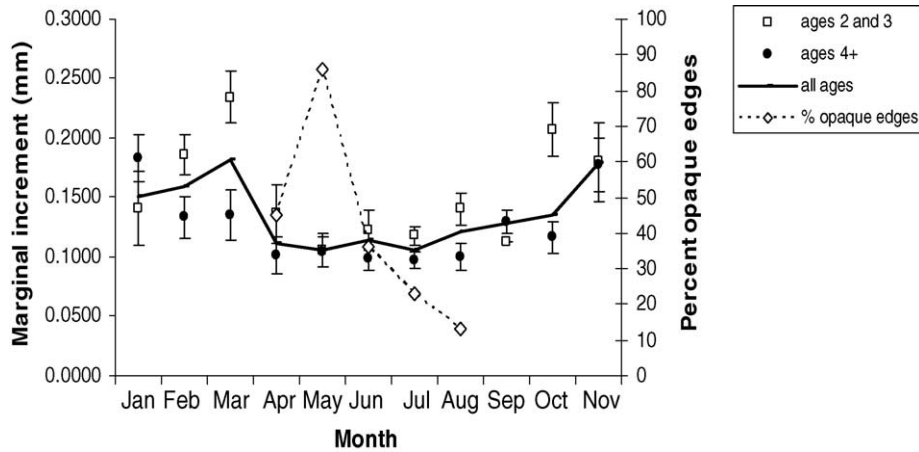


Fig. 6. Mean marginal increment (MIA)  $\pm$  one standard deviation and percent opaque edges (dashed line) by month from archived selected samples.

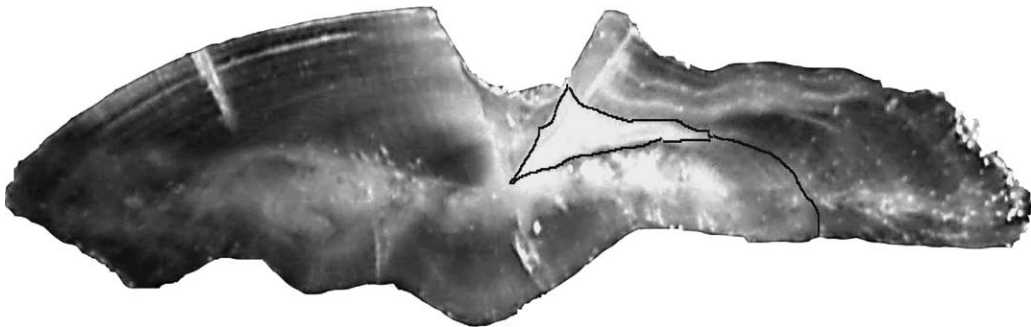


Fig. 7. The growth zone of an otolith (outlined by solid line) reflecting a broad first opaque area which includes the presumptive first annulus.

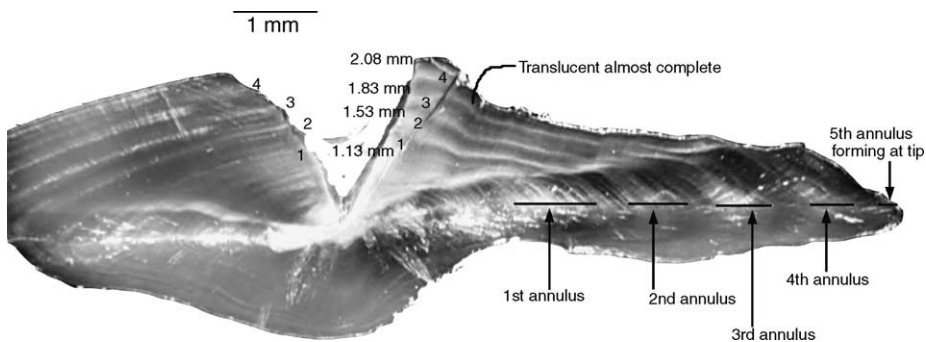


Fig. 8. Illustration of the relationship of the first annulus to subsequent annuli. The presumed first annulus is evident as a broadly diffuse opaque zone.

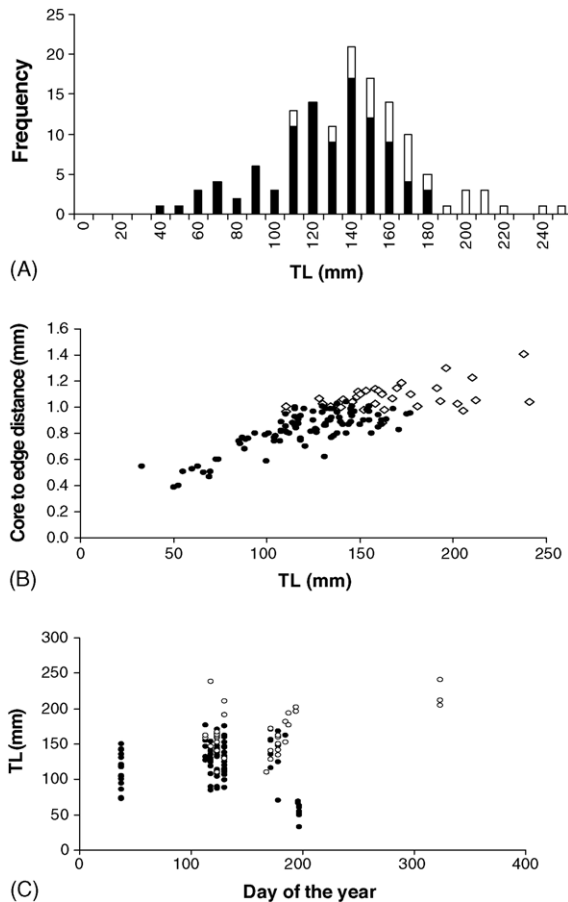


Fig. 9. (A) Length distribution (TL mm) of juvenile red snapper examined through the period of presumed first annulus formation (B) length (TL mm) and distance from the core to the otolith edge (mm) for the same juveniles with no annulus and one annulus visible and (C) length (TL mm) by day of year. Dark bars and symbols indicate an annulus was not visible, while open bars and symbols indicate an annulus was observed.

reader who over-counted annuli in young fish and under-counted the annuli in old fish (i.e., age > 8). Slight differences in otolith preparation between laboratories could have also been a factor in the reader differences. In addition, one would expect laboratories with the fewest otolith readers to be at a disadvantage since there would be fewer readers to detect and discuss incorrect ages. A separate, second reference collection with fewer old fish, which was more typical of the ages seen by laboratory 7 yielded an APE of 7.6% when compared to our laboratories' ages. Other studies have reported precision estimates from 3.7 to 8% APE

(Wilson and Nieland, 2001; Allman et al., 2002). Our results underscore the importance of a reference collection as a crucial quality control tool which must be used continuously to ensure that individual reader ages do not change over time and that ages from different readers remain consistent (Campana, 2001).

#### 4.2. Opaque zone formation

Our edge measurements revealed that there was a relatively sudden appearance of the opaque zone, a minimum marginal increment from April to July, and then a gradual increase in the width of the translucent zone through winter. Our results are broadly consistent with results across taxonomic and geographical boundaries; that is, the opaque zone is complete by spring to summer. The conclusion that this is an annual pattern is also consistent for the juvenile fish we examined. The pattern of sudden transition between translucence and opacity, the relatively short duration of opacity (one to a few months), followed by gradual increase in the width of the translucent zone is also consistent with a tropical pattern. The more visually distinct opaque zone is chosen to be counted as the annulus (Fowler, 1995).

The rates of transition from translucent to opaque were fairly consistent among other studies of red snapper (Fig. 11). However, we did note that there were differences in the seasonal timing of otolith zone transition on the order of a few months. The only other study performing edge measurements also revealed a sudden formation of the opaque zone, denoted by a minimal marginal increment, but the transition occurred between April and July (Futch and Bruger, 1976) as opposed to March and May for this study. By their result, peak frequency of opaque edges would have been expected in July. Several of the studies that utilized edge frequency analysis noted high proportions of opaque edges in February, March and April (Fig. 11). Together, these results suggest that the peak occurrence in opaque zone occurrence could range from March through July, while our results show peak opacity occurring during the midpoint of this range (i.e., May). These differences may occur due to interpretation error since MIA is largely subjective (Beckman and Wilson, 1995; Campana, 2001). In addition, annual or regional differences could have accounted for the shifts in timing of opaque-to-translucent zone formation. Some investigators have suspected or shown evidence that cooler temperatures



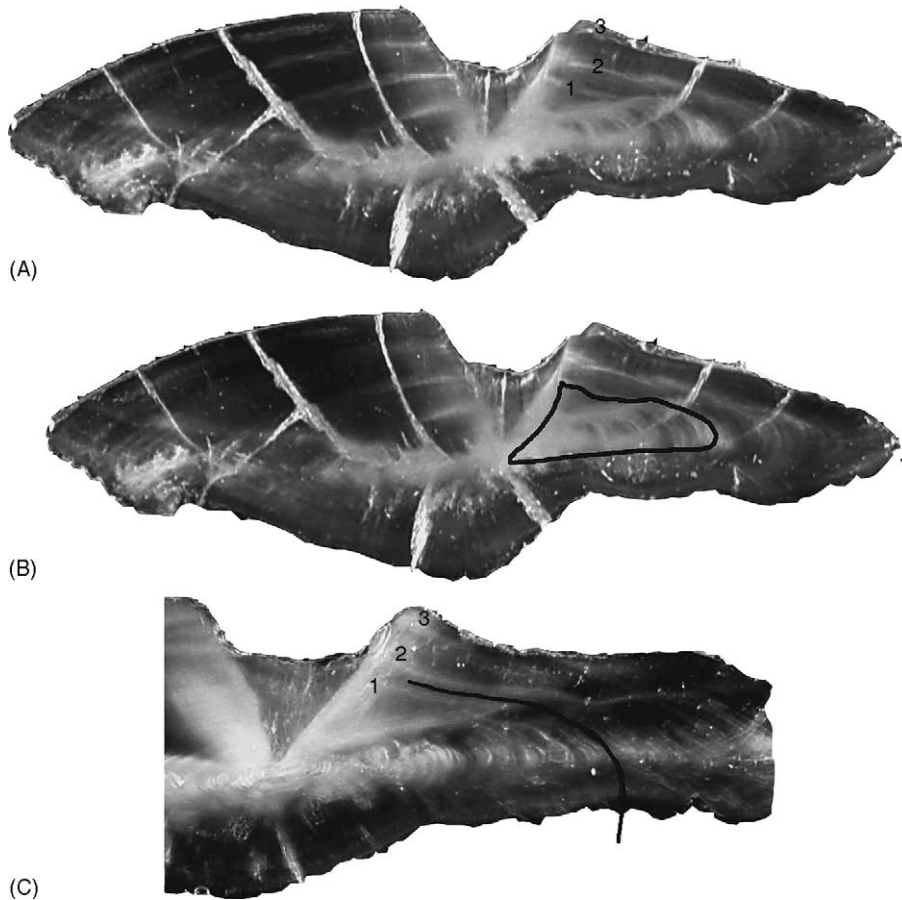


Fig. 10. These otolith sections have a count of three opaque zones enumerated as annuli, with number 3 just forming on the edge. The outlined area (B) indicates the broad first opaque zone but also shows an area of translucence between the core and the edge of the first annulus (A and B are same otolith). This pattern can give the false impression that four annuli are present. (C) More closely shows the delineation (solid line) between the first broad opaque zone and the second opaque zone.

can delay opaque zone formation (Beckman and Wilson, 1995; Pearson, 1996; Thomas, 1984; Smith and Deguna, 2003).

#### 4.3. First annulus formation

Red snapper commonly have a broad and diffuse first annulus which has also been noted in tropical lutjanids and other reef fishes (Fowler, 1995). Because red snapper experience a protracted spawning season (May–September) (Collins et al., 1996), Wilson and Nieland (2001) hypothesized that a greater distance from the core to the first annulus and the presence of translucence before the first annulus is completed sig-

nal a hatch date early in the spawning season, while an annulus close to the core suggests a hatch date late in the spawning season. This hypothesis was also repeated in a recent aging manual (Vanderkooy and Guindon-Tisdell, 2003) to explain the different patterns observed. While a test of this hypothesis was beyond the scope of this study, we did note that there was variation in the degree of opacity of the first annulus (compare Figs. 8 and 10). This may be a common trend among tropical to sub-tropical species (Fowler, 1995). However, we did not note much variation in the distance from the core to the distal edge of the first annulus. This distance was consistently about 1 mm.

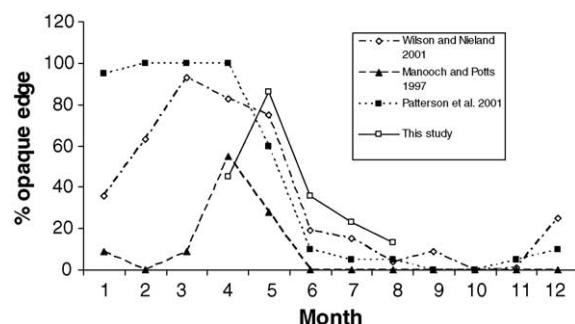


Fig. 11. Percentage of opaque edges in red snapper otoliths per month. Three studies measured the percent of opaque edges per month. Data from Wilson and Nieland (2001) and Manooch and Potts (1997) were interpolated from graphs similar to this one. Data from Patterson et al. (2001) were interpolated from a bubble plot and 2 years were aggregated.

We compared juvenile fish length at annulus formation with results of other studies as a check on our interpretation of the first annulus. We measured the total body length of fish sampled through the spring and summer when they were forming their first annulus based on our interpretation. We noted that the first annulus was forming in fish 110–180 mm TL at about 1 year following the expected peak spawning time (convention assumes a spawning date of 1 July; Patterson et al. (2001), based on Collins et al. (1996) and Szedlmayer and Conti (1999)). This agrees with previous estimates of size at first annulus, with modes of 110 mm TL in June and 130 mm TL in July (Holt and Arnold, 1982). In addition, the size range of 40–230 mm TL for first-year cohorts sampled from February to December in the 1970s (Holt and Arnold, 1982) matches our size range for the cohort sampled over a similar time frame in 2002. Szedlmayer and Conti (1999) reported that age-0 red snapper (no annulus observed) ranged up to 124 mm SL which is also consistent with the range that we observed.

#### 4.4. Implications for precision and accuracy of aging

Knowing when a species is expected to complete an annulus is important in assigning the fish to the correct year class. This is particularly crucial for a production aging approach used to characterize the age structure within a fishery, since fish are often sampled throughout

the year. If the timing of annulus completion varies, either geographically or inter-annually, it can affect a reader's ability to assign the correct age (Smith and Deguna, 2003; Pearson, 1996).

Red snapper follow a typical spring-summer pattern of annulus formation, but timing varies by at least a few months based on the various red snapper studies examined. Currently, our aging formula advances fish expected to complete an annulus from January through July which is consistent among all studies. After July, we expect opaque zone formation to be complete and ring count to be equal to age. This is a common approach and one that has been recommended in recent aging manuals (Panfili et al., 2002; Vanderkooy and Guindon-Tisdell, 2003). However, the period following expected annulus completion is clearly the period when age assignment is most uncertain (Smith and Deguna, 2003) and has been termed the "edge interpretation problem" (Francis et al., 1992). One recommendation may be to target sampling for annual age-structure during the period when opaque zone formation is complete (i.e., late summer through fall). However, restriction of sampling to certain times of year requires the assumption that the sampling period represents the age structure for fish collected at other times (e.g., no seasonal change in selection for age).

The often variable appearance of the first annulus has been one of the leading causes of reader disagreements during exchanges of red snapper otoliths. We found size at annulus formation to be consistent among studies and this gives us reason to believe that we are interpreting the first annulus correctly. The 1-mm distance from core to distal edge of first opaque zone may therefore be a good guideline for the expected annulus position and aid in interpretation. A carefully designed otolith marking study, using wild juveniles representing a range of spawning dates and possibly incorporating temperature variables, would further clarify interpretation problems. We feel that making consistent interpretations will elucidate the possible influence of differential spawning time and annual temperature/climatic signals.

#### Acknowledgments

We wish to thank Linda Lombardi-Carlson for help with data set organization and to Dan Foster, Butch Pellegrin and Ian Workman for collection of juvenile

red snapper. Will Patterson and Pete Sheridan provided helpful suggestions. We also wish to thank Dave Donaldson and Steve VanderKooy of the Gulf States Marine Fisheries Commission and the personnel from cooperating laboratories for taking part in the reference collection study. Additional support was provided by the National Marine Fisheries Service MARFIN (Marine Fisheries Initiative) and Red Snapper Research Programs. Otolith training workshops were funded by FIN (Fisheries Information Network).

## References

- Allman, R.J., Lombardi-Carlson, L.A., Fitzhugh, G.R., Fable, W.A., 2002. Age structure of red snapper (*Lutjanus campechanus*) in the Gulf of Mexico by fishing mode and region. *Gulf and Caribbean Fisheries Institute* 53, 482–495.
- Baker Jr., M.S., Wilson, C.A., 2001. Use of bomb radiocarbon to validate otolith section ages of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico. *Limnol. Oceanogr.* 46, 1819–1824.
- Beamish, R.J., Fournier, D.A., 1981. A method for comparing the precision of a set of age determinations. *Can. J. Fish. Aquat. Sci.* 38, 982–983.
- Beamish, R.J., McFarlane, G.A., 1995. A discussion of the importance of aging errors, and an application to walleye pollock: the world's largest fishery. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.), *Recent Developments in Fish Otolith Research*. University of South Carolina Press, Columbia, SC, pp. 545–565.
- Beckman, D.W., Wilson, C.A., 1995. Seasonal timing of opaque zone formation in fish otoliths. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.), *Recent Developments in Fish Otolith Research*. University of South Carolina Press, Columbia, SC, pp. 27–43.
- Bortone, S.A., Hollingsworth, C.L., 1980. Aging red snapper, *Lutjanus campechanus*, with otoliths, scales and vertebrae. *North-east Gulf Sci.* 4, 60–63.
- Campana, S.E., Annand, M.C., McMillan, J.I., 1995. Graphical and statistical methods for determining the consistency of age determinations. *Trans. Am. Fish. Soc.* 124, 131–138.
- Campana, S.E., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *J. Fish Biol.* 59, 197–242.
- Collins, L.A., Johnson, A.G., Kiem, C.P., 1996. Spawning and annual fecundity of the red snapper (*Lutjanus campechanus*) from the northeastern Gulf of Mexico. *ICLARM Conf. Proc.* 48, 174–188.
- Cowan Jr., J.H., Shipp, R.L., Bailey IV, H.K., Haywick, D.W., 1995. Procedure for rapid processing of large otoliths. *Trans. Am. Fish. Soc.* 124, 280–282.
- Crone, P.R., Sampson, D.B., 1998. Evaluation of assumed error structure in stock assessment models that use sample estimates of age composition. In: Funk, Quinn, Heifetz, Ianelli, Powers, Schweigert, Sullivan, Zhang (Eds.), *Fishery Stock Assessment Models*. University of Alaska Sea Grant College Program AK-SG-98-01, pp. 355–370.
- Fowler, A.J., 1995. Annulus formation in otoliths of coral reef fish—a review. In: Secor, D.H., Dean, J.M., Campana, S.E. (Eds.), *Recent Developments in Fish Otolith Research*. University of South Carolina Press, Columbia, SC, pp. 45–63.
- Francis, R.I.C.C., Paul, L.J., Mulligan, K.P., 1992. Ageing of adult snapper (*Pagrus auratus*) from otolith annual ring counts: validation by tagging and oxytetracycline injection. *Aust. J. Mar. Freshwater Res.* 43, 1069–1089.
- Futch, R.B., Bruger, G.E., 1976. Age, growth and reproduction of red snapper in Florida waters. In: Bullis Jr., H.R., Jones, A.C. (Eds.), in *Proceedings of the Colloquium on snapper-grouper fishery resources of the western central Atlantic Ocean*. Florida Sea Grant College Program Report No. 17, Gainesville, FL, pp. 165–183.
- Holt, S.A., Arnold, C.R., 1982. Growth of juvenile red snapper *Lutjanus campechanus*, in the northwestern Gulf of Mexico. *Fish. Bull. U.S.* 80, 644–648.
- Jearld Jr., A., 1983. Age determination. In: Nielsen, L.A., Johnson, D.L. (Eds.), *Fisheries Techniques*. American Fishery Society, Bethesda, Maryland, USA, pp. 301–324.
- Manooch Jr., C.S., Potts, J.C., 1997. Age and growth of red snapper, *Lutjanus campechanus*, Lutjanidae, collected along the southeastern United States from North Carolina through the east coast of Florida. *J. Elisha Mitchell Soc.* 113, 111–112.
- Morison, A.K., Robertson, S.G., Smith, D.C., 1998. An integrated system for production fish aging: Image analysis and quality assurance. *N. Am. J. Fish. Manage.* 18, 587–598.
- Nelson, R.S., Manooch Jr., C.S., 1982. Growth and mortality of red snappers in the west-central Atlantic Ocean and northern Gulf of Mexico. *Trans. Am. Fish. Soc.* 111, 465–475.
- Panfili, J., de Pontual, H., Troade, H., Wright, P.J., 2002. *Manual of fish sclerochronology*. Ifremer-IRD Coedition, Brest, France, p. 464.
- Patterson Jr., W.F., Cowan Jr., J.H., Wilson, C.A., Shipp, R.L., 2001. Age and growth of red snapper, *Lutjanus campechanus*, from an artificial reef area off Alabama in the northern Gulf of Mexico. *Fish. Bull. U.S.* 99, 617–627.
- Pearson, D.E., 1996. Timing of hyaline-zone formation as related to sex, location, and year of capture in otoliths of the widow rockfish, *Sebastes entomelas*. *Fish. Bull. U.S.* 94, 190–197.
- Smith, K.A., Deguna, K., 2003. Formation and annual periodicity of opaque zones in sagittal otoliths of *Mugil cephalus* (Pisces: Mugilidae). *Mar. Freshwater Res.* 54, 57–67.
- Szedlmayer, S.T., Conti, J., 1999. Nursery habitats, growth rates, and seasonality of age-0 red snapper *Lutjanus campechanus* in the northeast Gulf of Mexico. *Fish. Bull. U.S.* 97, 626–635.
- Thomas, R.M., 1984. A method of age determination for the south west African pilchard *Sardinops ocellata*. *S. Afr. J. Mar. Sci.* 2, 63–70.
- Vanderkooy, S., Guindon-Tisdell, K., 2003. A practical handbook for determining the ages of Gulf of Mexico fishes. Gulf States Marine Fisheries Commission. Publication Number 11. Ocean Springs, MS.
- Wilson, C.A., Nieland, D.L., 2001. Age and growth of red snapper, *Lutjanus campechanus*, from the northern Gulf of Mexico off Louisiana. *Fish. Bull. U.S.* 99, 653–664.